Long-Distance Singlet Energy Transfer along α-Helical Polypeptide Chains

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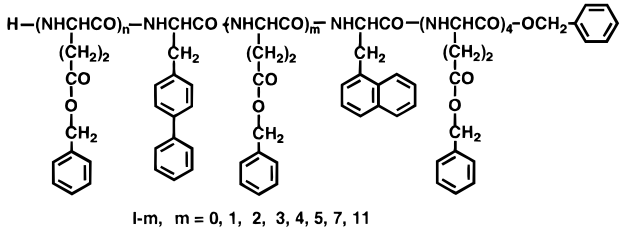
α-Helical polypeptides carrying L-4-biphenylalanine and L-1-naphthylalanine that are separated by up to 11 amino acid units or 19.5 Å were prepared by stepwise peptide synthesis. Singlet energy transfer from the biphenyl group to the naphthyl group was investigated with steady-state fluorescence spectroscopy, and the efficiency of energy transfer was evaluated as a function of the number of spacer units between the two nonnatural amino acids. The observed efficiency showed qualitative agreement with theoretical values calculated from interchromophore distances that were predicted from the helix geometry. When the biphenyl−naphthyl pair is separated by a single amino acid, higher transfer efficiency than that predicted by theory was observed. The high efficiency was interpreted in terms of contributions from higher multipole interactions. An effect of a phenyl group inserted between the donor and acceptor was also studied. The intervening phenyl group slightly enhanced the energy transfer when the aromatic rings of the donor, mediator, and acceptor groups were nearly in a face-to-face configuration.

Singlet excitation energy transfer (EnT) along a polypeptide chain has been studied by a number of workers.[1,2] The primary goal of these studies is to confirm the distance dependence of EnT that has been predicted to follow the Förster's r^{-6} rule. The rigid and well-defined helical structure of synthetic polypeptides is advantageous for the study of the distance dependence. Most workers have been using synthetic polypeptides carrying energy donors and energy acceptors covalently attached to the both ends. A common problem involved in these samples is that the termini of oligopeptides are more or less fluctuated and the end-to-end distance is distributed over some range that is difficult to evaluate experimentally and theoretically. Oligoproline chains have been often employed as the molecular framework, probably because of their synthetic versatility.[1] But oligoproline chains are not as stable as α-helix and take several different conformations depending on chain length, solvents, and other conditions.[3] The flexibility of the oligoproline chains leaves some uncertainty on the observed distance dependence.

In this study we have incorporated an energy donor and acceptor along an α-helical part of a long poly(γ-benzyl L-glutamate) chain. An α-helical main chain is advantageous because it is commonly found in protein structures and is stable in solution due to intramolecular hydrogen bonds. The chromophores were incorporated along the α-helix in the form of non-natural amino acids with aromatic side groups, L-4-biphenylalanine (B) and L-1-naphthylalanine (N). A common form of the present polypeptide samples is written as G_nBG_m-NG_4 (I-m, m = 0, 1, 2, 3, 4, 5, 7, 11).



In some cases, a γ-benzyl L-glutamate (G) unit was replaced with an alanine (A) unit, to minimize possible steric conflicts caused by racemization associated with a fragment condensation. By putting different numbers of amino acids between B and N units, the interchromophore distance can be varied periodically according to the geometry of the helix conformation.

It must be noted that the four G units at the C-terminal and the G_n units ($n \approx 30-40$) at the N-terminal play an essential role in stabilizing α-helical conformation of the sequence containing B and N units. Indeed, CD spectroscopy showed nonhelical conformations for oligopeptides such as Boc-BG_m-NG_4 (Boc = *tert*-butyloxycarbonyl).

Another advantage of α-helical polypeptide chain as the molecular framework is that a third component may be inserted between the donor and acceptor, without changing the donor−acceptor distances and their orientations substantially. In this study, two types of polypeptides that carry a phenylalanine (F) unit between the B and N units were prepared. One of the polypeptide has a sequence of NFB (II), and the other has a sequence of NG_2FGAB (III). As will be described later, the former polypeptide has the triad chromophore sequence that is almost perpendicular to the helix and the latter has the triad sequence that is almost parallel to the helix.

These samples were used to examine possible roles of intervening aromatic groups on the EnT. Although EnT and its distance dependence have been used to measure biological distances, little has been known as to the possible roles of aromatic groups located on the pathway of EnT. The study of EnT on the above model for polypeptides may clarify the possible mediation effects of aromatic residues.

Experimental Section

Synthesis of Polypeptides. Polypeptides were prepared by a two-step procedure. First Boc-oligopeptides that contains the sequence of the B−N pair and a G_4 unit at the C-terminal (Boc-BG_mNG_4) were prepared by stepwise synthesis through a liquid-phase or solid-phase method. For long Boc-oligopeptides such as Boc-$BG_5AG_5NG_4$, fragment condensations of short peptides such as Boc-BG_5A-OH and H-G_5NG_4 were carried out. Racemization of up to a few percent has been known to occur at

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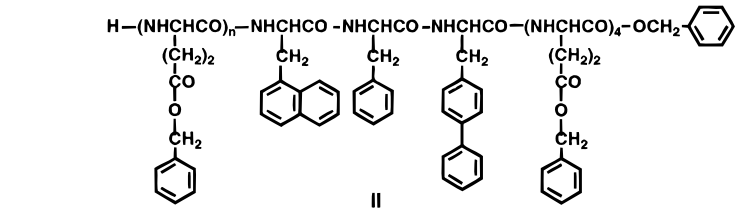

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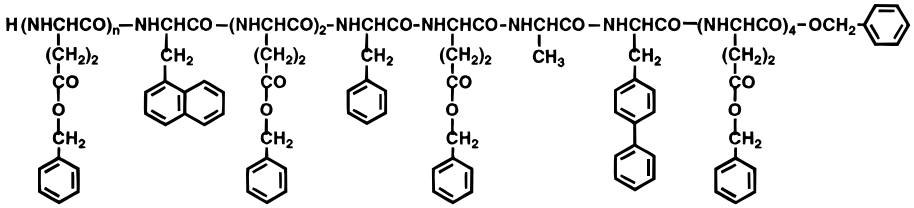

III

the C-terminal unit of the N-fragment during the fragment condensation. To reduce the effect of the racemization, an alanine unit was chosen as the C-terminal of the N-fragment because the small side group of the alanine unit will minimize the steric perturbation on the helical conformation.

The Boc-oligopeptides were purified with preparative HPLC until single peaks were obtained and characterized by ^{1}H NMR. The Boc group was then removed with 3 N HCl in dioxane and neutralized by washing with sodium bicarbonate solution. The oligopeptides with free amino terminals were used as initiators for polymerization of γ-benzyl L-glutamate *N*-carboxyanhydride (G-NCA) in dimethylformamide (DMF). The resulting polypeptides were fractionated with a Sephadex LH-60 column in DMF to exclude low-molecular weight-fractions, if they were found.

In the following section, procedures for preparing Boc-oligopeptides are described. More detailed procedures have been described before on the synthesis of polypeptides that contain electron donors and acceptors.[4]

Liquid-Phase Synthesis of Peptides. *Boc-NG$_4$-OBzl and Boc-BG$_4$-OBzl.* Boc-G$_4$-OBzl[4] was deprotected with 4 N HCl/dioxane and coupled with Boc-N-OH or Boc-B-OH with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride/1-hydroxybenzotriazole hydrate/triethylamine (EDC·HCl/HOBt/TEA) in DMF. The reaction mixture was evaporated, and the remaining oil (or solid) was redissolved in ethyl acetate. The latter solution was washed with 10% citric acid, 10% NaCl, 4% $NaHCO_3$, and 10% NaCl solution and evaporated under reduced pressure. The product was recrystallized from ethyl acetate/ether and isolated as colorless crystals. ^{1}H NMR (500 MHz) in $CDCl_3$ showed 5 different C$^\alpha$H protons.

Boc-NBG$_4$-OBzl, Boc-FBG$_4$-OBzl, and Boc-ABG$_4$-OBzl. The pentapeptide Boc-BG$_4$-OBzl was deprotected with 4 N HCl/dioxane and coupled with the Boc derivative of the N-terminal amino acid (Boc-N-OH, Boc-F-OH, or Boc-A-OH) through the same procedure as above. The product was recrystallized from ethyl acetate/ether and isolated as colorless solid.

Boc-NABG$_4$-OBzl and Boc-NFBG$_4$-OBzl. The corresponding Boc-hexapeptide (Boc-ABG$_4$-OBzl or Boc-FBG$_4$-OBzl) was deprotected and coupled with the corresponding Boc-amino acid through the same procedure as above. The product was recrystallized from ethyl acetate/ether. ^{1}H NMR (500 MHz) of Boc-NABG$_4$-OBzl in $CDCl_3$ showed seven different C$^\alpha$H protons.

Solid-Phase Synthesis Using Oxim Resins. Oxim resins were prepared by a standard procedure.[5] The resin was first charged with Boc-G-OH (0.71 mmol/g of resin). The extension of peptides was carried out as follows. First the Boc protecting group was removed by a 25% trifluoroacetic acid/dichloromethane (TFA/DCM) mixture for 20 min. The Boc derivative of the next amino acid (3-fold excess) was mixed in DMF containing BOP reagent, HOBt, and diisopropylethylamine (DIEA). The DMF solution was gently shaken with the resin for 30 min at 25 °C and washed with DMF and DCM. The Kaiser test was carried out to detect amino groups remaining in the resin, and if the result was negative, the next Boc-amino acid was attached.

Cleaving Peptides from Oxim Resins. The peptides on oxim resins were cleaved off by two different procedures. When Boc-peptide benzyl ester was a desired product, the peptide was cleaved off by putting a 3-fold excess of TosOH·G-OBzl, DIEA, and acetic acid into the resin suspended in DMF. The mixture was gently stirred at 25 °C for 24 h and filtered. The filtrate was concentrated, and the peptide was precipitated by adding 10% citric acid. After the peptide was dried under vacuum, it was redissolved in ethyl acetate and precipitated in ether. The peptide was fractionated with preparative HPLC.

When a Boc-peptide with a free C-terminal was needed for the N-terminal peptide of fragment condensation, the peptide was cleaved from the resin by mixing a DMF solution of a 3-fold excess of hydroxypiperidine. The mixture was gently stirred at 25 °C for 24 h and filtered. The filtrate was concentrated and redissolved in acetic acid. To the latter solution was added a 5-fold excess of $Na_2S_2O_4$ in H_2O, and the mixture was stirred at 25 °C for 1 h. The solution was concentrated, and the peptide was precipitated by adding water. The peptide was fractionated with preparative HPLC.

Preparative HPLC. Reverse-phase preparative HPLC was carried out with a Millipore μ Bondasphere 5μ C$_4$ 100 Å (∅ 19 × 150 mm) column eluted with 0.1% TFA/water with a gradual addition of acetonitrile from 50% to 100%.

Fragment Condensation. Long Boc-oligopeptides such as Boc-BG$_5$AG$_5$NG$_4$-OBzl that are denoted as Boc-peptide$_1$-peptide$_2$-OBzl were synthesized by condensation of Boc-peptide$_1$-OH with H-peptide$_2$-OBzl. Each fragment was prepared by a liquid-phase or solid-phase method followed by preparative HPLC. The Boc-peptide$_1$-OH was designed to have a C-terminal alanine (A) unit in order to minimize conformational conflicts due to partial racemization at the C-terminal unit during the fragment condensation. The fragment condensation was carried out for equimolar mixture of Boc-peptide$_1$-OH and H-peptide$_2$-OBzl in the presence of EDC·HCl and HOBt in DMF. The mixture was stirred at 0 °C for 2 h and subsequently at 25 °C for 22 h. The resulting peptide was treated as usual and fractionated with preparative HPLC until a single peak was obtained.

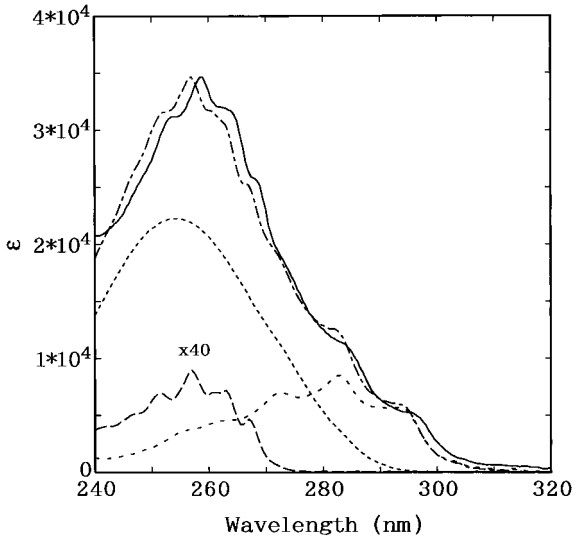

Figure 1. Absorption spectrum of the polypeptide $G_nBG_2NG_4$ in trimethyl phosphate and its resolution into components of naphthyl (- - -), biphenyl (---), and phenyl (— — —) chromophores. The component spectra were taken from spectrum of Boc-L-1-naphthylalanine, Boc-L-*p*-biphenylalanine, and Boc-L-phenylalanine, respectively. The relative weights of the component spectra are 1:1:40 for naphthyl:biphenyl:phenyl chromophores, respectively.

Attachment of the Long Glu(OBzl) Chain to the Oligopeptide. Each Boc-oligopeptide was treated with 4 N HCl/dioxane or 25% TFA/DCM to remove the Boc group and neutralized by shaking the peptide in chloroform with 4% $NaHCO_3$ solution. After drying in vacuum, the peptide was weighed and dissolved in DMF. A 40-fold amount of G-NCA was added and polymerized at 25 °C for 48−72 h. The completion of the polymerization was checked by the disappearance of the characteristic IR peaks of NCA. The polypeptide was precipitated in ether, washed with ether, and fractionated on a Sephadex LH60/DMF column to remove low-molecular-weight fractions, if they are present.

Characterization of Polypeptides. Polypeptides were characterized by absorption spectroscopy. As a typical example, the spectrum of $G_nBG_2NG_4$ is shown in Figure 1 (solid line). The spectrum was resolved into contributions from N, B, and benzyl group, as follows. First, the amount of the N group was determined by the absorbance at 295 nm and the spectrum contribution of the N group was taken from the absorption spectrum of Boc-N (small dash line). The contribution of the B group was taken from the spectrum of Boc-B (dot line). The amount of the B unit was, in this case, assumed to be the same as that of N unit. Finally the amount of the benzyl group was determined to give the best fit between the combined spectrum of the three components (dash−dot line) and the observed spectrum of the polypeptide. In the case of BG_2NG_4, the number of benzyl groups that gives the best fit to the observed spectrum was 40 ± 4. The relatively large uncertainty is caused by the small absorption coefficient of the phenyl group in this region. The combined spectrum is shown by a dash−dot line in Figure 1. Although the observed spectrum of the polypeptide showed a small red shift compared to the combined spectrum, the total profile indicates that the polypeptide contains an equal number of N and B groups and about 40 benzyl groups. Since seven benzyl groups are already included in the oligopeptide BG_2NG_4, the average number of G units at the N side must be $n = 33 \pm 4$.

The observed spectrum profile sometimes deviated from the sum of equimolar amounts of N and B units. In these cases, the numbers of B and benzyl groups were determined by a least-squares fitting of the observed spectrum to the sum of the component spectra. The results are listed in Table 1. In most cases, the B/N ratios are close to unity, indicating that the two chromophores are correctly incorporated in the polypeptides. The number average degrees of polymerization of the G_n units are large enough to hold stable α-helical conformations of the polypeptides.

TABLE 1: Characterization of Polypeptide Samples, $Glu(OBzl)_n$-(chromophore sequence)-$Glu(OBzl)_4$

chromophore sequence[a]	n[b]	[B]/[N][c]
B	25	
N	48	
NB	29	1.02
BG_1N	43	1.04
NAB	41	1.01
NFB	36	1.04
BG_2N	33	1.00
BG_3N	34	1.00
BG_4N	47	1.06
BG_5N	22	1.01
BG_4AG_2N	31	0.99
BG_3FAG_2N	44	1.00
BG_2AG_5N	42	1.04
BG_5AG_5N	31	1.07

[a] B = L-biphenylalanine, N = L-1-naphthylalanine, F = L-phenylalanine, A = L-alanine, G = γ-benzyl L-glutamate. [b] Number of Glu(OBzl) units on the left-hand side of polypeptides. The estimated error is ±10%. [c] Molar ratio of biphenyl to naphthyl groups determined from least-squares resolution of the absorption spectrum.

Methods

UV, CD, and fluorescence spectra were measured in trimethyl phosphate that is transparent down to 190 nm. Fluorescence spectra were measured under an argon atmosphere. The following spectrometers were used: absorption, Jasco Ubest 55; CD, Jasco J500; steady-state fluorescence, Jasco FP777. The output of each spectrometer was interfaced to a NEC PC9801 computer.

Molecular mechanics calculations of the synthetic polypeptides containing several non-natural amino acids were made on a software PEPCON.[6] The structure and energy parameters of PEPCON were taken from the ECEPP system.[7] Molecular models were drawn with a PC version of NAMOD (version 3).[8]

Results and Discussion

Conformation and Chromophore Arrangement of Polypeptides. UV absorption spectra of the polypeptides were essentially the sum of B and N chromophores, indicating that the chromophores are correctly incorporated in the polypeptides and that they are electronically isolated from each other in the ground state.

The CD spectrum of each polypeptide was measured in TMP solution. The CD spectrum in the amide absorption region (190−240 nm) showed a typical pattern of an α-helix for all the polypeptides examined. As a typical example, the CD spectrum of $G_nBG_2NG_4$ is shown in Figure 2. The $\Delta\epsilon$ value at 222 nm was in the range -12 ± 2 for all polypeptides, indicating stable α-helical conformations. The relatively large scattering of $\Delta\epsilon$ values is again caused by the difficulty in determining the number of G units in G_n.

CD spectra at the aromatic absorption region showed complex patterns depending on the chromophore sequences. In the case of $G_nBG_2NG_4$, for example, a broad positive peak was observed for the biphenyl group and a small negative peak was detected

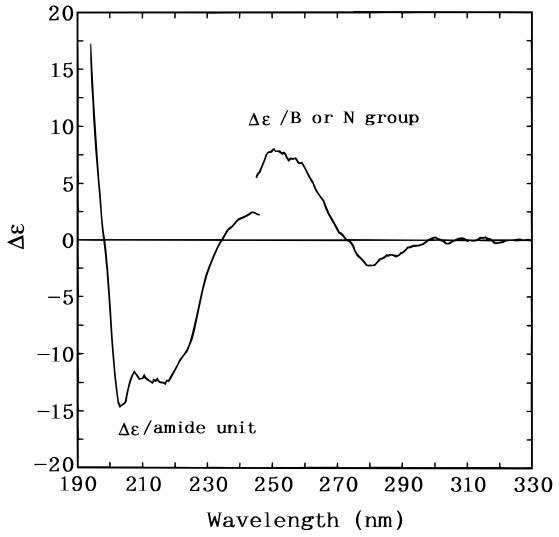

Figure 2. Circular dichroic spectrum of the polypeptide $G_nBG_2NG_4$ in trimethyl phosphate.

in the absorption region of the naphthyl group. The observation of CD peaks suggests that the chromophores are not freely rotating around the $C^{\alpha}-C^{\beta}$ (χ_1) and $C^{\beta}-C^{\gamma}$ (χ_2) bonds but are restricted within a narrow range of the rotational angles.

Molecular mechanics calculations were carried out to predict side-chain orientations, assuming an α-helical main chain. All glutamate units were replaced by alanine units, for simplicity. There were two possible orientations for a naphthyl group, one being with (χ_1,χ_2) = (180°,65°) (type I) and the other with (180°,265°) (type II). The two energy minima have almost equal energies and equal allowed areas in the (χ_1,χ_2) energy contour map. Therefore, two equally possible center-to-center biphenyl−naphthyl distances exist for each biphenyl−naphthyl pair. The distances are listed in Table 2. The computer-predicted conformations of the polypeptide that contains a B−N pair separated by 11 amino acids are shown in Figure 3.

In the case of the polypeptide G_nNFBG_4 that contains an NFB sequence, the N−B distance is predicted to be essentially the same as that in the polypeptide G_nNABG_4. The computer-predicted conformation of G_nNFBG_4 is shown in Figure 4. Since there is an enough space between the N and B groups, the insertion of an F group will not change the N−B distance very much. Similarly, the B−N distance of the polypeptide G_nBG_3-FAG_2NG_4 is predicted to be the same as that of the polypeptide $G_nBG_4AG_2NG_4$. The computer-predicted conformation of the former polypeptide is shown in Figure 5. For the two polypeptides carrying an F unit between B and N groups, we may discuss the effect of the phenyl group on the EnT under the condition of the same interchromophore distances and orientations of biphenyl and naphthyl groups.

Steady-State Fluorescence Spectra and the Efficiency of Energy Transfer. Fluorescence spectra of the polypeptides were measured in trimethyl phosphate solution. The excitation wavelength (275 nm) was chosen so as to minimize excitation of phenyl groups attached on the side chain. At this excitation wavelength, 58% of the photoenergy is absorbed by the biphenyl group and 42% by the naphthyl group. The fluorescence spectrum of a polypeptide carrying a BG_5N sequence is shown in Figure 6. In the same figure, results of least-squares decomposition of the spectrum into fluorescence spectra of B and N groups are shown by dashed lines. Energy transfer efficiencies were evaluated from the intensity of B fluorescence (I_B) and that of N fluorescence (I_N). These intensities are expressed in terms of the absorption coefficients of the B and

TABLE 2: Center-to-Center Distances and Energy Transfer Efficiencies for the Biphenyl−Naphthyl Pair on α-Helical Polypeptides, $Glu(OBzl)_n$-(chromophore sequence)-$Glu(OBzl)_4$

chromophore sequence	no. of spacer amino acids	orientation of N unit[a]	r_{cc}[b] (Å)	E_{ent} (calcd)[c]	E_{ent} (obsd)[d]
NB	0	I	9.9	0.92	0.96
		II	11.8	0.80	
BG_1N	1	I	12.8	0.71	0.90
		II	13.6	0.64	
NAB	1	I	13.7	0.63	0.86
		II	13.5	0.64	
NFB	1	I	13.7	0.63	0.84
		II	13.6	0.64	
BG_2N	2	I	6.4	0.99	0.96
		II	8.8	0.96	
BG_3N	3	I	6.7	0.99	0.95
		II	4.7	1.0	
BG_4N	4	I	14.0	0.60	0.61
		II	13.4	0.65	
BG_5N	5	I	13.1	0.68	0.51
		II	14.5	0.54	
BG_4AG_2N	7	I	14.0	0.60	0.61
		II	12.8	0.72	
BG_3FAG_2N	7	I	13.5	0.64	0.66
		II	12.5	0.74	
BG_2AG_5N	8	I	17.6	0.27	0.37
		II	17.9	0.25	
BG_5AG_5N	11	I	20.2	0.14	0.25
		II	19.6	0.16	

[a] Type I orientation: (χ_1,χ_2) = (180°,65°). Type II orientation: (180°,265°). [b] Center-to-center distance between B and N groups in Å. [c] Theoretical efficiency using r_0 = 14.9 Å. [d] Observed efficiency from steady-state fluorescence spectrum.

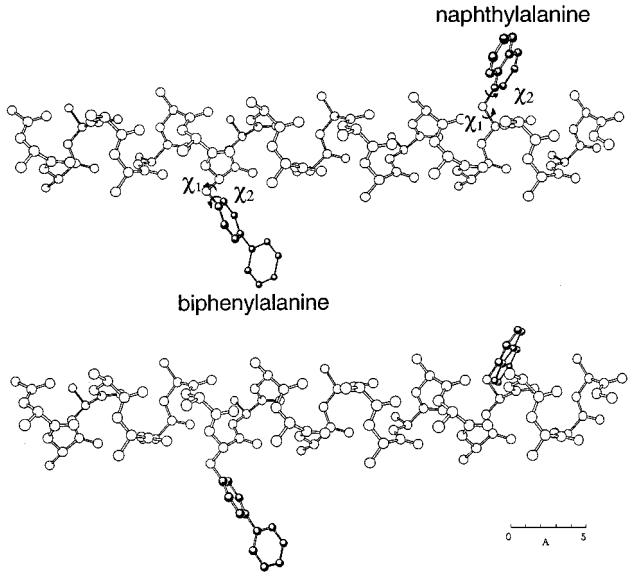

Figure 3. Computer-predicted conformation of the polypeptide $A_{16}BA_{11}NA_4$: (top) type I orientation of naphthyl group; (bottom) type II orientation.

N groups (ϵ_B = 9180 and ϵ_N = 6650) at 275 nm, quantum yields of B and N fluorescence (η_B and η_N), concentrations of B and N groups ([B] and [N]), and the EnT efficiency (E_{ent}):

$$I_B = (\text{instrumental factor})\eta_B\epsilon_B[B](1 - E_{ent}) \quad (1)$$

$$I_N = (\text{instrumental factor})\eta_N(\epsilon_N[N] + \epsilon_B[B]E_{ent}) \quad (2)$$

Setting [B] = [N], the I_N/I_B ratio can be expressed as

$$I_N/I_B = \eta_N(\epsilon_N + \epsilon_B E_{ent})/\eta_B\epsilon_B(1 - E_{ent}) \quad (3)$$

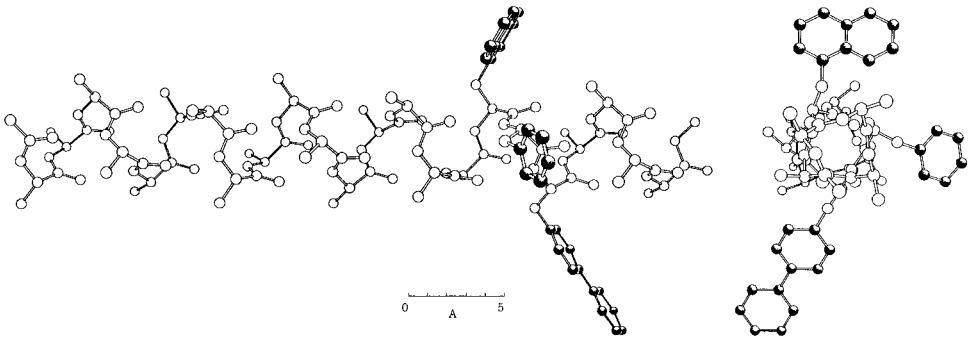

Figure 4. Computer-predicted conformation of the polypeptide $A_{16}NFBA_4$. Orientation of the naphthyl group is type I.

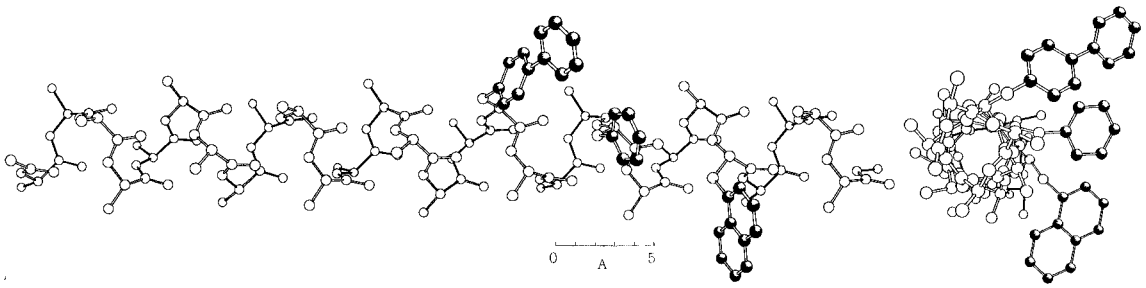

Figure 5. Computer-predicted conformation of the polypeptide $A_{16}BA_3FA_3NA_4$. Orientation of the naphthyl group is type I.

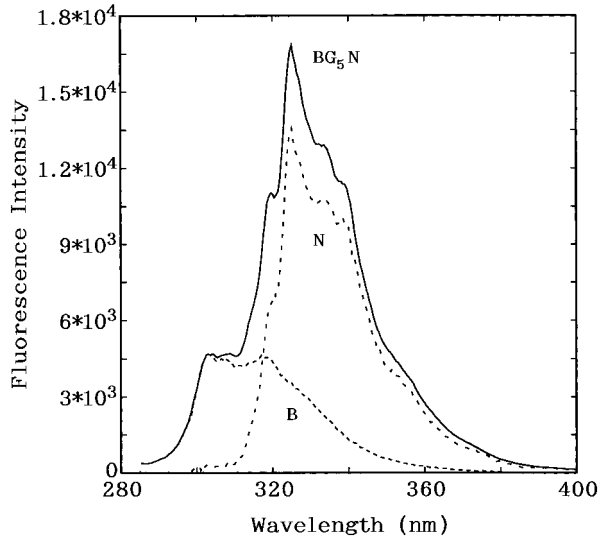

Figure 6. Fluorescence spectrum of the $G_nBG_5NG_4$ polypeptide in trimethyl phosphate. The results of least-squares resolution into B and N components are also shown. Fluorescence spectra of G_nBG_4 and G_nNG_4 polypeptides are used as the component spectra for B and N groups, respectively.

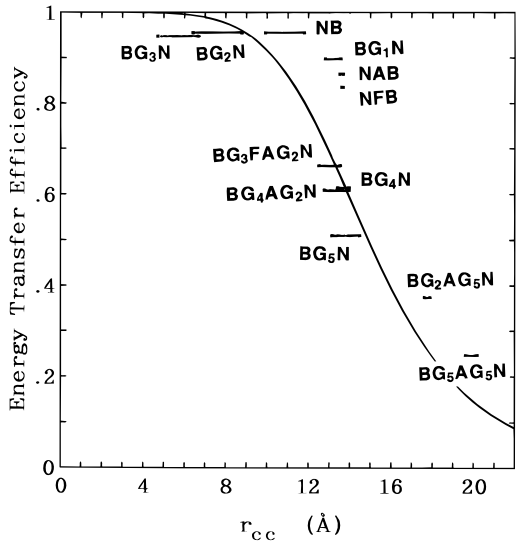

Figure 7. Energy transfer efficiency plotted against the center-to-center distance between B and N groups, r_{cc}. Experimental data are plotted against r_{cc}'s that are predicted from molecular mechanics calculations. Since there are two types of naphthyl orientations, the r_{cc} values have some uncertainties that are expressed by horizontal bars. The solid curve represents theoretical values according to Förster's r^{-6} rule with $r_0 =$ 14.9 Å.

The η_N/η_B ratio was obtained from the fluorescence intensity of each polypeptide that contains a single B group or single N group (η_N(325 nm)/η_B(302.5 nm) = 0.687, λ_{ex} = 275 nm). Using eq 3, the EnT efficiencies were calculated and the results are listed in Table 2. The EnT efficiencies are plotted against the center-to-center B−N distances in Figure 7. Since there are two possible orientations for a naphthyl group, the possible ranges of the inter-chromophore distance are shown by horizontal bars.

Figure 7 also shows a theoretical curve calculated from the Förster's r^{-6} theory:

$$E_{ent} = r_0^6/(r_0^6 + r^6) \qquad (4)$$

The critical distance of EnT from an excited biphenyl to a naphthyl group, r_0, was calculated from an overlap integral of biphenyl fluorescence and naphthyl absorbance and the fluorescence quantum yield of biphenyl (0.143) to be 14.9 Å.

Most of the data points lie near the theoretical curve, indicating that the EnT obeys the r^{-6} law on α-helical polypeptides and that the chromophores are correctly spaced up to 20 Å along the α-helix as predicted from conformational calculations. The steep distance dependence clearly demonstrates that thermal fluctuations of the α-helix are of minor importance even at room temperature.

It must be argued that the EnT rates should also depend on the relative orientation of the two chromophores. The above discussion presumes that the orientations are dynamically averaged to give $\kappa^2 = {}^2/_3$. For the evaluation of orientation factors, the direction of transition moment for fluorescence emission of a biphenyl group and that for absorption of a

naphthyl group are needed. The biphenyl fluorescence is originated from a vibronic mixing of an electronic transition polarized along the long axis with a variety of vibrational modes. Therefore, its fluorescence band consists of many differently polarized vibronic peaks.[9] Furthermore, the absorption band of the naphthyl group in the 285 nm region is assigned to a ^{1}La band that is electronically polarized along the short axis. However, this band is only partially allowed [oscillator strength $\approx$ 0.05 (calculated[10]) and 0.13 (observed for 1-methylnaphthalene)] and its polarization depends on the mode of coupling in each vibronic peak. Therefore, the orientation factor of EnT from an excited biphenyl to a naphthyl group is preaveraged and may not sharply depend on the relative orientation of the two chromophores. In the present study, as a first approximation, the orientational factor for dynamic average ($^2/_3$) was assumed for all polypeptides that possess different relative orientations of the two chromophores.

Highly Efficient Energy Transfer in BXN and NXB Sequences. Fairly large deviations were found for three polypeptides that contain BG_1N, NAB, and NFB sequences. All the three polypeptides have the B−N pair separated by a single amino acid and show higher EnT efficiencies than those expected from the Förster theory. The authors believe that the high efficiencies are attributed neither to any problem in polypeptide samples nor any experimental error involved in fluorescence measurements, since the phenomenon is common to the polypeptides with BXN or NXB sequences and those polypeptides show normal absorption and CD spectra.

As illustrated in the case of the polypeptide G_nNFBG_4 in Figure 4, a common feature of the BXN or NXB polypeptide is that the edge-to-edge B−N distance is much shorter than the center-to-center distance. In the case of G_nNFBG_4, for example, the edge-to-edge distance is 9.4 Å for both type I and II orientations of the naphthyl group, that is shorter than the center-to-center distances, 13.7 and 13.6, for type I and II orientations, respectively. The EnT efficiency calculated according to the Förster theory is based on the interaction between point dipoles that are positioned on the centers of the B and N groups. If the B−N distance is shorter, contributions of higher multipole−multipole interactions will become more important and will increase the EnT efficiency. Furthermore, direct interactions between π orbitals of B and N groups including the Dexter's mechanism of EnT may enhance the EnT. This may explain the high EnT efficiencies of the three polypeptides. High efficiency of the polypeptide that contains the NB sequence is also explained in a similar manner. In the case of the polypeptides that contain BG_2N and BG_3N sequences, the center-to-center distances are already very small and the above consideration makes little sense.

Effect of Phenyl Group Lying between B and N Groups. One of the unknown factors in the singlet EnT is a possible effect of π orbitals that are inserted between the energy donor and acceptor. Although the superexchange mechanism on electron transfer has been discussed by a number of workers, the superexchange mechanism on EnT has been attracting very little attention. Recently, Ghiggino and co-workers[11] discussed theoretically the role of the superexchange mechanism in EnT. Experimental evidence of the superexchange effect is usually difficult to obtain, since we have to compare efficiencies of EnT in the presence and in the absence of an intervening aromatic group without changing other factors, such as donor−acceptor distance and orientation.

α-Helical polypeptides provide an ideal molecular framework on which energy donor, possible mediator, and acceptor groups are arranged with designed distances and orientations. Polypeptides that contain sequences of NAB and NFB were prepared for the study of the possible superexchange mediation of the F group. As expected from Figure 4, the spatial arrangement of the N−B pair may be kept unchanged by the insertion of a phenyl group, but the phenyl group is outside the line that connects the N−B pair. In this case, the EnT was not enhanced by the phenyl group. Indeed, the efficiency of the NFB polypeptide was slightly smaller than that of the NAB polypeptide.

To examine the effect of the phenyl group that is lying on the B−N line, polypeptides that contain sequences of BG_3FAG_2N and BG_4AG_2N were synthesized. In both polypeptides, the B−N pairs are separated by seven spacer amino acids, but the former has a phenylalanine unit in the middle of the spacer. The computer-predicted conformation of the BG_3FAG_2N polypeptide is shown in Figure 5. The center-to-center distances between the B−N pair in the BA_3FA_3N sequence are predicted to be 14.0 and 12.8 Å for type I and II orientations of the N group, respectively. The corresponding distances in the BA_7N sequence are 13.5 and 12.5 Å. The center-to-center distances between B and F groups in the BA_3FA_3N sequence are 5.9 Å for the two naphthyl orientations, and the center-to-center distances between F and N groups are 7.7 and 6.6 Å for types I and II, respectively. The calculation suggested that the B−N distance is very slightly enlarged by the insertion of the phenyl group.

The EnT efficiencies of the two polypeptides are compared in Table 2 and in Figure 7. The efficiency increased by 5% by the insertion of a phenyl group just on the line of the B−N pair. The enhancement, although very small, may be attributed to some electronic effect, since it is unlikely to expect that the B−N distance becomes shorter by the replacement of a γ-benzyl L-glutamate unit by a phenylalanine unit. Absorption spectra of the two polypeptides were the same, and the positions of fluorescence peaks were not shifted at all. These observations suggest that the small enhancement may be caused by a superexchange mechanism that involves virtual states of the phenyl π system. Since the enhancement is very small, however, a modest conclusion is that a very small mediation of EnT, if it exists actually, may be observed when donor, mediator, and acceptor groups are arranged in a face-to-face configuration.

Conclusions

A variety of α-helical polypeptides that contain a pair of B and N groups were synthesized. Energy transfer efficiency from steady-state fluorescence measurements followed the Förster theory up to 20 Å separation of the B−N pair. The polypeptides that contain BXN and NXB sequences showed higher EnT efficiency than those expected from the theory, indicating a contribution of higher multipole−multipole interactions. The phenyl group in the NFB sequence showed no evidence of enhancing EnT. The phenyl group in the BG_3FAG_2N sequence showed a slight enhancement of EnT, suggesting the presence of the superexchange mechanism.

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